REMARKS

Status of the Claims

This paper amends claim 1. Claims 9-14 and 21-46 are canceled without prejudice. Applicants reserve the right to prosecute canceled subject matter in related applications. After the amendments set forth above are entered, claims 1-6 and 8 and 15-16 are pending and under examination.

Support for Amendments

Support for the amendments to claim 1 is found in claim 13 as originally filed.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1, 3-6, 8-13, and 15-16 stand rejected under 35 U.S.C. § 112, first paragraph for both inadequate written description and lack of enablement. For clarity, each rejection is address separately below.

Written Description

Claims 1, 3-6, 8-13, and 15-16 stand rejected for inadequate written description. Specifically, the Examiner alleges that the claims and specification do not adequately describe "pathway components of a TGF-β signaling pathway" and "cell fate-inducing polypeptides".[1] Applicants respectfully traverse these rejections.

Claim 1 is amended herewith to specify that the TGF-β signaling pathway component is Smad4. Smad4 is a well-known cellular protein and Applicants describe Smad4$^{-/-}$ embryonic

[1] Office Action mailed January 11, 2007 at page 3, fourth paragraph.

stem cells and provide experimental results using same.[2] Thus, this rejection should be withdrawn to the extent that it applies to the TGF-β signaling pathway component.

Turning next to the alleged inadequate description of cell fate-inducing polypeptides, the Examiner asserts that the specification neither provides distinguishing features or attributes concisely shared by the members of the genus,[3] nor does the specification teach a representative number of species of this genus.[4] Applicants respectfully disagree.

The specification defines "cell fate-inducing polypeptides" as those protein transcription factors that cause pluripotent cells to differentiate into cell types of the nervous system (e.g., neurons and glia) and identify such proteins as transcription factors.[5] This description is sufficient to distinguish the genus of "cell fate-inducing polypeptides" by features and attributes shared by its members.

Applicants also identify five specific examples of cell fate-inducing genes and their effects on pluripotent cells. Applicants note that Nurr-1 and PTX-3 are involved in the development of dopaminergic neurons,[6] whereas Phox2a regulates development of nor-adrenergic neurons. Shh2 is identified as regulating the development of dopaminergic and serotonergic neurons. And, AP2 generally regulates genes involved in catecholamine production. These exemplary members induce at least three different neuronal cell types: dopaminergic, serotonergic, and adrenergic. The identification of these five transcription factors is also sufficient to fully describe the metes and bounds of the genus by teaching a representative number of species of the genus.

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[2] See, Specification at page 39, Example 1.

[3] Office Action mailed January 31, 2007 at page 3, fourth paragraph.

[4] Office Action mailed January 31, 2007 at page 4, first paragraph.

[5] Specification at page 7, line 14 through page 8, line 20.

[6] Specification at page 7, line 14 through page 8, line 20, and page 18, line 17 through page 20, line 6.

Furthermore, the skilled artisan immediately understands what is meant by this terminology because the prior art recognizes a variety of other genes which encode "cell-fate inducing polypeptides". For example, Lee et al. note that Pax2, Pax5, Wnt1, and En1 also "control differentiation of dopaminergic and serotonergic neurons in the midbrain and hindbrain."[7]

In sum, the specification provide a definition of "cell fate-inducing polypeptides" which specifically identifies distinguishing features shared by the genus. The specification also describe a representative number of members (five) that fall within the genus. Furthermore, the prior art clearly recognizes a variety of additional genes which cause pluripotent cells to differentiate into cell types of the nervous system. Thus, the term "cell fate-inducing polypeptides", as used in the instant claims and specification, is clearly recognized and understood by the skilled artisan. Applicants respectfully submit that this rejection is traversed and should be withdrawn.

Enablement

Claims 1, 3-6, 8-13, and 15-16 stand rejected for lack of enablement. Specifically, the Examiner alleges that the specification does not reasonably enable methods for generating dopaminergic neurons in vitro or in vivo comprising inhibiting one or more pathway components of a TGF-β signaling pathway in any pluripotent target cell and overexpressing one or more cell fate-inducing polypeptides in the target pluripotent cells.[8] Applicants respectfully traverse these rejections.

Applicants first point out that the Federal Circuit has made clear that a patent need not reiterate techniques known to skilled workers in a particular area of technology. See Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986); In re Wands, 858 F.2d 731, 8USPQ2d 1737 (Fed. Cir. 1987) ("A patent need not teach, and preferably omits,

[7] Nature Biotechnol., 18: 675-679, 2000. See page 675, right column, second paragraph.

[8] Office Action mailed January 11, 2007 at paragraph bridging pages 4-5.

what is well known in the art); see also Paperless Accounting, Inc. v. Bay Area Rapid Transit Sys., 804 F.2d 659, 231 USPQ 649 (Fed. Cir. 1986) ("A patent applicant need not include in the specification that which is already known to and available to the public.").

The amount of direction or guidance presented in the specification and the presence or absence of working examples..

The Examiner alleges that the specification provides insufficient guidance to enable the claimed invention.[9] As an initial matter, Applicants note that the claims are amended herewith to require that the dopaminergic neurons are generated in vitro and that the TGF-β signaling pathway component is Smad4. Thus, to the extent that this rejection applies to the in vivo production of dopaminergic neurons and/or the inhibition of TGF-β signaling pathway component generally, the rejection is rendered moot.

The specification and the prior art enable the skilled artisan to practice the full scope of the currently amended claims without the need for undue experimentation. The claimed method generates dopaminergic neurons from pluripotent cells in vitro by (i) inhibiting Smad4 and (ii) overexpressing one or more cell fate-inducing polypeptides. Applicants provide examples of this method.

In Example 1, the specification describes an in vitro culture of embryonic stem cells (pluripotent cells) that are $Smad4^{-/-}$.[10] Furthermore, the specification demonstrates these pluripotent $Smad4^{-/-}$ cells progressively lose mesodermal markers and gain certain general neuronal markers and dopaminergic markers.[11] Thus, the specification provides a working example of the inhibition of Smad4 in pluripotent cells and characterizes certain properties of those cells in order that they may be identified and further used by the artisan without the requirement for undue experimentation.

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[9] Office Action at pages 8-9.

[10] Specification at pages 39-45.

[11] Specification at page 43, line 21 through page 44, line 5, and Figures 3-4.

The specification provides alternate methods for inhibiting Smad4 in pluripotent cells. Specifically, the specification also provides for the use of a Smad4 dominant negative protein[12] and the use of Smad4 antisense nucleic acids.[13] A C-terminal truncation of Smad4 (e.g., Smad4(1-514)) results in dominant negative protein, as taught by Simeone et al.,[14] Chen et al.,[15] and Zhang et al.[16] Effective Smad4 antisense nucleic acids are taught by Kretschmer et al.[17] and Zhao et al.[18] Thus, the specification provides direction for multiple methods of Smad4 inhibition which, in view of common knowledge in the prior art, fully enable the in vitro inhibition of Smad4.

Turning next to the requirement for overexpression of one or more cell fate-inducing polypeptides, Applicants point out that the in vitro overexpression of genes is generally well-known in the art. Specifically relevant to the instant claims, the specification exemplifies a method for the stable transfection of cell fate-inducing polypeptides.[19] This method subclones a nucleic acid encoding a cell fate-inducing polypeptide into a standard vector, amplifies and purifies the vector from *E. coli*, and then transfects embryonic stem cells using lipofectamine. The specification also incorporates by reference other prior art methods for overexpressing cell-fate inducing polypeptides (and other polypeptides) in pluripotent cells.[20]

In sum, the specification provides a great deal of guidance for practicing the claimed invention. The specification provides a working example of the use of pluripotent cells in which

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[12] Specification at page 30-31.

[13] Specification at pages 24-26.

[14] Am. J. Physiol. Cell Physiol. 281: C311-C319, 2001. See, page C313 "Construction of Adenoviral Vectors" and Figure 7.

[15] J. Biol. Chem. 277: 36118-36128, 2002. See paragraph bridging pages 36119-36120.

[16] Am. J. Physiol. Gastrointest. Liver Physiol., 280: G1247-G1253, 2001. See page G1248, "Construction of Adenoviral Vectors".

[17] Oncogene, 22: 6748-6763, 2003. See page 6761, "GB Antisense Oligonucleotides" and Figure 6C.

[18] Dev. Biol., 194: 182-195. See paragraph bridging pages 183-184 and Figures 2 and 4.

[19] Specification at page 31, line 22 through page 33, line 15.

[20] Specification at page 31, lines 9-21.

a TGF-β signaling pathway component is inhibited and guides the artisan on methods for using these cells in conjunction with the overexpression of a cell fate-inducing polypeptide. Thus, when the specification and the prior art are viewed together, it is clear that the specification proves ample guidance for a skilled artisan to practice the full scope of the claimed invention without undue experimentation. Accordingly, for this reason alone, this rejection is traversed and should be withdrawn.

State of the prior art and the predictability or unpredictability of the art.

The Examiner alleges that the prior art demonstrates that the state of the art of gene therapy and stem cell development in organisms is unpredictable.[21] Specifically, the Examiner alleges that Branch and Crooke teach that the in vivo application of nucleic acids is highly unpredictable and that cell culture examples are generally not predictive of in vivo inhibition of target genes. The Examiner also alleges that Agrawal et al., Peracchi, and Opalinska et al. document the technical difficulties and unpredictability associated with gene therapy and achieving in vivo efficacy using nucleic acid-based approaches.

Applicants note that the instant claims are amended herein to specify only in vitro processes. Thus, this basis for rejection (i.e., the unpredictability of in vivo gene therapy and associated processes) is moot.

The Examiner also alleges that the prior art demonstrates differences in the use of human and murine ES cells. Specifically, the Examiner notes that Zwaka et al. state that high stable transfection efficiencies in human ES cells are difficult to achieve and that Odorico et al. document differences for in vitro culture requirements for maintaining the various ES cells.

Applicants respectfully disagree with the Examiner's characterization of the unpredictability of the prior art as it relates to the in vitro use of murine and human ES (and other pluripotent) cells and submit that the Examiner's reading of Zwaka et al. is unduly narrow.

[21] Office Action at pages 5-8.

Applicants first point out that the mere inefficiency for transfecting human ES cells is not an appropriate basis for a lack of enablement rejection. In the statement relied upon by the Examiner, Zwaka et al. refer to transfecting human ES cells using protocols that had been established for mouse ES cells. Zwaka et al. discloses the mouse ES cell protocols yielded a stable transfection rate of about 10^{-7} in human ES cells. Although Zwaka suggests that murine-optimized electroporation protocols work poorly in human ES cells and that higher transfection rates are desirable, nowhere does Zwaka suggest that electroporation of human ES cells is unworkable. Thus, for this reason alone, Zwaka fails to support the lack of enablement rejection and in fact demonstrates that the claimed method is enabled.

Zwaka also demonstrates that, by performing nothing more than routine experimentation, the murine electroporation protocols may be optimized for human ES cells and higher yields obtained. Specifically, Zwaka et al. state that

> [a]s human ES cells are significantly larger than mouse ES cells (~14 μm versus ~8 μm), we tried electroporation parameters described for larger cells. Additionally, we electroporated the cells in an isotonic, protein-rich solution (standard cell culture medium), instead of PBS, at room temperature. Using this modified protocol, we were able to obtain stable... transfection rates that were 100-fold (or more) higher than those attained with standard mouse ES cell electroporation procedures. Zwaka et al. at page 1, right column, first paragraph (emphasis added).
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> [e]lectroporation of human ES cells with a DNA construct containing a neo cassette under the control of the *tk* promoter yielded a stable transfection rate of 5.6×10^{-5}, giving an estimated 26:1 ratio of stable transfected clones to homologous recombination events for the first POU5F1 construct. Similarly, for transfection of the HPRT1 vector, the ratio of G418-resistant clones to HPRT1$^-$ clones was 50:1. Zwaka et al. at page 2, first paragraph (internal citations omitted) (emphasis added).

Zwaka, using standard techniques known in the art at the time of application filing, easily modified the murine-optimized electroporation protocols to increase transfection efficiency in human ES cells.

In sum, Zwaka et al. demonstrate that the results of murine-optimized electroporation protocols are successful, albeit inefficient, when applied to human ES cells. Zwaka et al. further demonstrate that the protocols are easily improved by routine experimentation using techniques known in the art for transfection of larger ES cells (i.e., human). Accordingly, when read in its entirety, Zwaka et al. prove that genetically modified human ES (pluripotent) cells can be created in vitro and also prove that Applications' specification fully enables the claims as currently amended.

Turning next to Odorico et al., Applicants note that, to the extent that this reference is relied upon to demonstrate the unpredictability providing therapy using ES (or other pluripotent) cells, the basis for rejection is rendered moot by the current claim amendments. With respect to the Examiner's implication that Odorico et al. somehow demonstrate unpredictability in culture of human ES cells, Applicants assert the contrary. Odorico et al. provide discussion of variables related to the successful culture of human ES cells. For example, Odorico et al. state:

> The [inner cell mass] cell outgrowths are propagated in the presence of serum and colonies with the appropriate undifferentiated morphology are subsequently selected and expended. After the initial derivation in serum, human ES cell lines can be maintained and propagated on feeder layers in medium containing serum alone or serum replacement medium and basic fibroblast growth factor (bFGF). Odorico et al., at page 194, left column.

Thus, Odorico et al., like Zwaka et al., provide significant information regarding the in vitro features of pluripotent human cells. Both of these references, contrary to the Examiner's allegation, support the predictability of Applicants' claimed invention and the prior art.

The breadth of the claims and the quantity of experimentation required.

The Examiner further alleges that the claims are overly broad and that the specification fails to provide sufficient guidance to the skilled artisan without the requirement for undue experimentation.[22] Applicants respectfully disagree with this allegation but note that the claims

[22] Office Action at page 9.

are amended herein to require that the dopaminergic neurons are generated in vitro and that the TGF-β signaling pathway component is Smad4. In view of these amendments and the foregoing arguments, Applicants respectfully submit that practicing the invention as currently claimed does not require undue experimentation and that this rejection should be withdrawn.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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